Exhibit A

MEMORANDUM

DATE: March 13, 1989

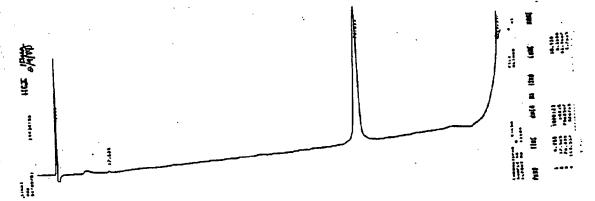
TO: Tom Boone & Keith Langley

FROM: Patricia Fausset and Hsieng Lu Vice

RE: N-terminal Sequence Analysis of Recombinant Human Collagenase Inhibitor (HCI) (Sample #020389B).

1. HPLC of rHCl:

One sample of HCI (0.5mg/ml) was submitted to this laboratory for N-terminal sequence analysis. Appr. 10ug of the sample was loaded directly onto a Protein C4 column and run on RP-HPLC. The chromatogram generated is shown below:



The result indicates that the sample chromatographed as a single, symmetrical peak at retention time of 78.8min. which elutes at appr. 41%B mobile phase (in 90% CH₃CN and 0.1% TFA).

- 2. N-terminal sequence analysis:

 Appr. 500 pmol of the sample was loaded onto a sequencer and the first 18 amino acids were sequenced. Two sequences were detected:
- 1. Major Sequence (86%): (C)-S-(C)-S-P-V-H-P-Q-Q-A-F-(C)-N-A-D-V-V-...
- 2. Minor Sequence (14%): S-(C)-S-P-V-H-(X)-Q-(Q)-A-F-(C)-N-(X)-D-V-...

[where()=amino acid not positively assigned from sequencing]
The minor sequence indicates that the first cysteine at the Nterminus has been processed. No methionyl sequence is detected in
this sample.

3. C-terminal Analysis:

The protein was digested with carboxypeptidase P at 25 C and aliquots were taken at different time points (5, 10, 20, 40, 60, and 120 min.) and analyzed for amino acids released by the digestion. However, carboxypeptidase P poorly digested HCI. Many amino acids were released at poor rates and cannot be correlated well with the expected C-terminal sequence. Thus, the precise C-terminal sequence cannot be assigned from the experiment. However, all the C-terminal amino acids up to 14 residues from the C-terminal proline are present in the digest. To further confirm the intactness of the C-terminus for HCI, we suggest that isolation of C-terminal tryptic peptides should be performed.

cc: B. Altrock, L. Souza, D. Vapnek